Content

Purify transfection-grade plasmid DNA from bacteria using a fully automated, magnetic bead-based custom protocol with the Wizard MagneSil Tfx™ System on the CyBio FeliX liquid handler.

Automated Purification of Plasmid DNA using Promega Wizard MagneSil Tfx™ System on the Analytik Jena CyBio FeliX Liquid Handler

Application Summary

Kit: Wizard MagneSil Tfx™ System, 4 x 96 preps (Cat.# A2380)
Analyses: UV absorbance & agarose gel electrophoresis
Sample Type(s): Bacteria from overnight cultures
Input: 0.5 mL to 1 mL of bacterial culture

Materials and Methods

Instruments and Equipment

- CyBio FeliX Basic Unit with Enclosure (Cat.# OL5015-24-100)
- CyBio FeliX Extraction Set (Cat.# OL5015-25-120)
- Protective Plate, 532 Pieces (Cat.# OL3317-25-126)
- Nunc® 2.0 mL Deep-Well Plate Adapter (QInstruments, Cat.# 2016-1151)
- MagnaBot® FLEX 96 Magnetic Separation Device (Cat.# VA1290)

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Consumables and Reagents

- Wizard MagneSil Tfx™ System (Cat.# A2380)
- CyBio TipRack 96/1000 µL; PCR-certified, pre-sterilized, filter (Cat.# OL3811-25-939-F)
- Reservoir, 4 column, polypropylene, 73 mL (Agilent, Cat.# 201308-100)
- 2.0 mL Deep Well Plates (Sterile) (Cat.# AS9307)
- 100% Isopropanol (molecular biology grade)
- 80% Ethanol (molecular biology grade)

Methods

Sample Preparation

Aliquot 0.5 mL to 1 mL of cultured bacteria into 96 well plates (round bottom 2 mL deep well) and centrifuge at 4,500 x g for 30 minutes to pellet bacterial cells. Manually remove and discard the supernatant.

Wizard MagneSil Tfx™ System and Instrument Setup

- **Endotoxin removal plate** - Add 40 µL of Endotoxin Removal Resin to a new 96 well plate for each sample to be processed. Use the same well positions as the sample preparation plate.
- **Elution plate** - Add 100 µL of Nuclease-Free Water to a new 96 well plate for each sample to be processed. Use the same well positions as the sample preparation plate.
- **Binding mastermix preparation** – Mix 50 µL of MagneSil® RED and 435 µL of 100% isopropanol (plus a 10% overage) per sample into position 1 of a single 4-column reservoir.
- **Other kit reagents will be loaded to 4-column reservoirs according to the CyBio FeliX method instructions.** See Table 1 for reagent volumes used per reaction.

Table 1: Volumes used for the Wizard MagneSil Tfx™ System.

Purification Step	Reagent	Reservoir (4 column) or 96 well plate	Volume Used per Reaction
Lysate Clearing	Resuspension	Reservoir1, Column 1	115 µL
Lysate Clearing	Lysis	Reservoir1, Column 2	150 µL
Lysate Clearing	Neutralization	Reservoir1, Column 3	150 µL
Lysate Clearing	MagneSil® BLUE	Reservoir1, Column 4	30 µL
Endotoxin Removal	Endotoxin Removal Resin	96 well plate	40 µL
Binding & Wash	MagneSil RED	Reservoir2, Column 1	50 µL
Binding & Wash	100% Isopropanol	Reservoir2, Column 2	435 µL
Binding & Wash	Prepared 4/40 wash	Reservoir2, Column 3	250 µL
Binding & Wash	80% Ethanol (1/2)	Reservoir2, Column 4	250 µL
Binding & Wash	80% Ethanol (2/2)	Reservoir2, Column 4	250 µL
Elution	Nuclease-free Water	96 well plate	100 µL

Instrument Method

Load and run the CyBio FeliX method "Wizard MagneSil Plasmid R96-1000 (Endotoxin Removal).bms" and follow the on-screen instructions. Instrument and deck layout pictures are shown in Figure 1.

A

B

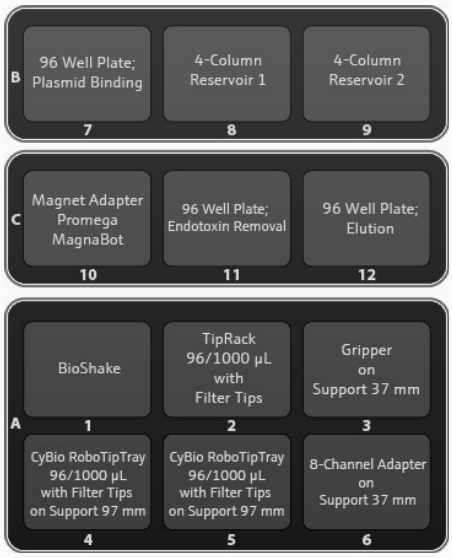

Figure 1: Deck layout for plasmid DNA purification on the CyBio FeliX Liquid Handler using the Wizard MagneSil Tfx™ System. Reagents are dispensed by the instrument from 4-column reservoirs, with the exception of Endotoxin Removal Resin and Elution Buffer which are pre-dispensed manually in the indicated deep well plates. **(A)** Analytik Jena CyBio FeliX instrument with the CyBio FeliX Extraction Set, the Promega MagnaBot® FLEX 96 Magnetic Separation Device and Bioshake 3000-T elm mounted with a Nunc® 2.0 mL heat plate adapter. **(B)** Reagent, labware, consumable and accessory positions for implementing the Wizard MagneSil Tfx™ System kit.

Results

Plasmid DNA purifications were performed using the method described above from 0.5 mL and 1 mL pelleted bacterial cultures. JM109 E. coli bacteria (Cat.# L2005) was transformed with pGL4.50 (Cat.# E131A) and cultured overnight in Terrific Broth medium (TB) or Luria Broth medium (LB). Replicate purifications were performed (n=23). Concentrations and absorbance ratios (A260/A280 and A260/A230) were determined using spectrophotometry (NanoDrop™ One). Plasmid size was visualized using gel electrophoresis. Plasmid DNA was successfully purified from both 0.5 mL and 1 mL of LB and TB cultured bacteria, respectively. As expected, extractions from TB culture resulted in higher plasmid yields due to the increased density of the overnight culture. Absorbance purity ratios and gel electrophoresis demonstrated that the purified plasmid DNA was of high quality and purity.

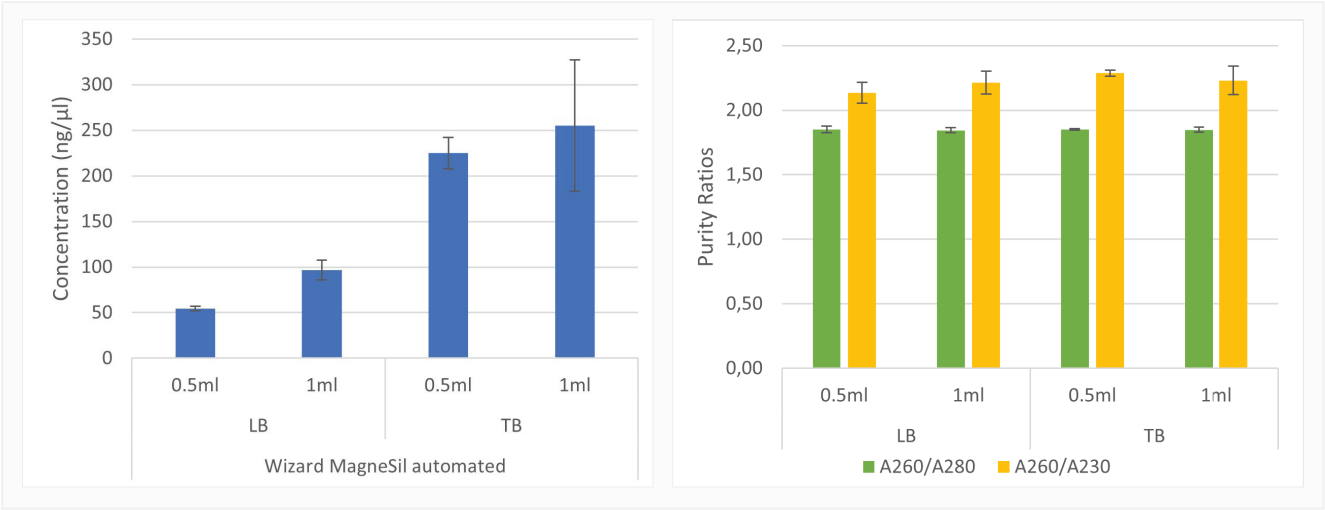

Figure 2: Concentrations and absorbance ratios of plasmid DNA purified from bacteria using the Wizard MagneSil Tfx™ System on the CyBio FeliX Liquid Handler.
Plasmid DNA was purified from both 0.5 mL and 1 mL overnight cultures in either TB or LB medium. DNA concentrations (left) and A260/A280 and A260/A230 absorbance ratios (right) were measured using a NanoDrop™ One spectrophotometer. Shown are the average values and standard deviations from n=23 replicate extractions.

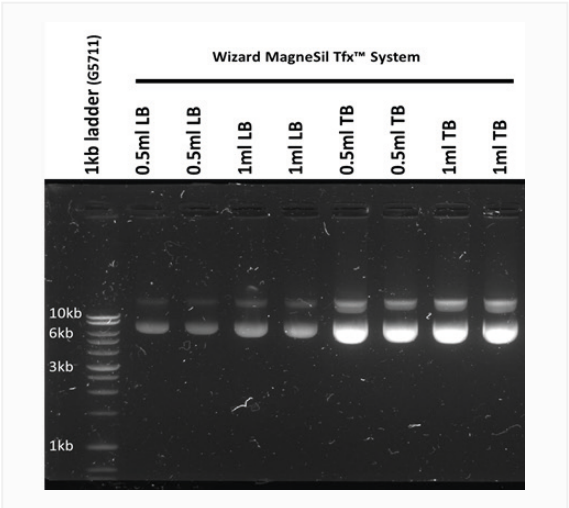

Figure 3: Agarose gel electrophoresis of plasmid DNA purified from bacteria using the Wizard MagneSil Tfx™ System on the CyBio FeliX Liquid Handler.
10 µL of purified plasmid (1 mL or 5 mL of overnight bacterial culture in either LB or TB medium, as indicated) were analyzed using a 0.8% agarose gel. A 1kb DNA Ladder (Cat.# G5711) was included as a size standard.

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